CLAIMS

1. Method for optically detecting at least one entity whereby
   - the at least one entity is arranged on and/or in a substrate (60), said substrate (60) preferably being arranged on a support (61) and having a refraction-index which is different from the one of an at least one component adjacent to the substrate (60),
   - the at least one entity is scanned with a measuring volume (70) using at least one device being confocal or configured for multi-photon-excitation said device consisting of a radiation source (10) and at least one objective (32), thereby receiving measuring values of optical parameters which are processed by means of signal processing for characterization of the at least one entity,
   - the at least one entity substantially maintains its position with respect to the substrate (60) or the support (61) or both for the duration of the obtaining the measuring values,
   - before and/or during the scanning process an auxiliary focus (71) is generated by means of at least one second radiation source (11) and an objective (34), said auxiliary focus (71) is at least partly arranged on the interface (62) between substrate (60) and adjacent component or on another interface (62) having a defined spacial relation to said entity,
   - a retroreflection from the auxiliary focus (71) is detected by a confocal arranged detector (21) or a plurality of detectors (21,22) being arranged in front of and/or behind the image-plane and along the optical axis of the optic (34) generating the auxiliary focus (71), said retroreflection is used for measuring the position of the interface (62) and, thus, for indirectly positioning the measuring volume (70), and
   - the position of the auxiliary focus (71) relative to the measuring volume (70) is adjusted or adjustable in a defined manner.

2. Method according to claim 1 characterized in that the auxiliary focus (71) is generated by means of the same radiation source (10) and/or the same objective (32) which also serves for generating the measuring volume (70).

3. Method according to claim 1 or 2 characterized in that the extent of the confocal detected volume of the auxiliary focus (71), in particular in direction of the respective optical axes of the objectives (32,34) is smaller than the extent of the measuring volume (70).

4. Method according to claim 3 characterized in that the auxiliary focus (71) for obtaining a smaller extent of the confocal detected volume of the auxiliary focus (71) is generated by an optic (34) having a numeric aperture which is larger than the numeric aperture of the optic (32) used for generating the measuring volume (70).

5. Method according to claim 3 characterized in that for obtaining the small extent of the confocal detected volume of the auxiliary focus (71) the smaller part of the numerical aperture of a common optic or the respective optics (32,34) is used for generating the measuring volume (70) than for generating the auxiliary focus (71).

6. Method according to claim 3 characterized in that for obtaining the small extent of the confocal detected volume of the auxiliary focus (71) a confocal arranged diaphragm (51) is used at the detection of the auxiliary focus (71), said diaphragm (51) having a smaller opening than a confocal arranged diaphragm (50) used at the detection of the measuring volume (70

7. Method according to claim 1 characterized in that for indirectly positioning the measuring volume (70) the position of the auxiliary focus (71) relative to the interface (62) is moved preferably periodically substantially along the optical axis of the optic (34) generating the auxiliary focus

(71), the intensity of the retroreflection in dependence on the movement of the detector (21) is registered and the position of the auxiliary focus (71) is readjusted in a manner that the intensity of the retroreflection reaches its maximum.

8. Method according to claim 1 characterized in that for indirectly positioning the measuring volume (70) the position of the auxiliary focus (71) relative to the interface (72) is moved both, laterally to the optical axis of the optic (34) generating the auxiliary focus (71) and axial.

9. Method according to claim 7 or 8 characterized in that the amplitude of the preferably periodical movement of the auxiliary focus (71) is smaller than or equal to the axial extent of the measuring volume (70).

10. Method according to claim 1 characterized in that the intensity of the retroreflection is detected by means of at least two detectors (21,22) and the position of the interface (62) is determined by means of the distribution of the intensities detected by the detectors (21,22).

11. Method according to claim 1 whereby the scatter-light-intensity and/or the scatter-light-intensity in dependence on the polarization and/or the fluorescence-intensity at at least one wavelength and/or the fluorescence-intensity in dependence on the polarization and/or the fluorescence-durability and/or molecular luminosity and/or Raman-scattering and/or luminescence are detected as optical parameters.

12. Method according to claim 1 whereby mineral or organic substrates (60) in particular polymeric gels, polymeric particles built up from inorganic materials, vesicular structures, cells, bacteria and virus are used.

13. Method according to claim 1 whereby molecules, molecule-complexes, polymers, polymeric particles, particles built up from inorganic material, vesicular structures, cells, bacteria and virus are used as entities.

14. Method according to claim 1 whereby entities and/or substrates (60) selected by means of the optical parameters are separated during or after the scanning process from the other entities and/or substrates (60).

15. Method according to claim 1 for use in the research of active ingredients, in the functional analysis of combinatoric-chemical or combinatoric-biological synthesis-products, in the functional genom-analysis, in the evolutive biotechnology, in the diagnostics, in the proteom-analysis or the investigation of material.

16. Apparatus for performing the method according to claim 1 comprising
   - at least one first radiation source (10) as well as at least one device being confocal or configured for multi-photon-excitation said device comprising an objective (32) and at least one first detector (20) for detecting measuring values from the measuring volume (70),
   - at least one second radiation source (11) as well as at least one further device comprising an objective (34) and at least one second detector (21) for detecting a retroreflection from an auxiliary focus (71) whereby a confocal arranged second detector (21) or a plurality of detectors (21,22) being arranged in front of and/or behind the image plane and along the optical axis of the optic (34) generating the auxiliary focus (71) are used,
   - at least one device for positioning measuring volume (70) and auxiliary focus (71) relative to a substrate (60), and
   - a device for variably positioning the auxiliary focus (71) relative to the measuring volume (70).

17. Apparatus for performing the method according to claim 1 comprising
   - at least one first radiation source (10) as well as at least one device being confocal or configured for multi-photon-excitation said device comprising an objective (32) and at least one first detector (20) for detecting measuring values from a measuring volume (70),

- at least one second radiation source (11) as well as at least one further device comprising an objective (34) in at least one second detector (21) for detecting a retroreflection from an auxiliary focus (71) whereby a confocal arranged second detector (21) or a plurality of detectors (21,22) arranged in front of and/or behind the image-plane and along the optical axis of the objective (34) generating the auxiliary focus (61) is used,
- at least one device for positioning of measuring volume (70) and auxiliary focus (71) relative to the substrate (60),
- whereby the auxiliary focus (71) is adjusted relative to the measuring volume (70) in a defined manner.

18. Apparatus according to claim 16 characterized in that the device for positioning the measuring volume (70) and the auxiliary focus (71) relative to the substrate (60) comprises means for positioning the auxiliary focus (71) relative to the measuring volume (70).

19. Apparatus according to one of the claims 16 or 18 characterized in that the same first radiation source (10) and/or objective (32) is used for generation of the measuring volume (70) and the auxiliary focus (71).

20. Apparatus according to claim 16 characterized in that the device for positioning the auxiliary focus (71) relative to the measuring volume (70) comprises means for adjusting the relative position of the objectives (32,34).

21. Apparatus according to claim 16 characterized in that the device for positioning the auxiliary focus (71) relative to the measuring volume (70) comprises means for variation of the convergence of those ray bundles being focussed by the respective objective (32,34) for generating the auxiliary focus (71) and the measuring volume (70).

22. Use of the apparatus according to claim 16 in the research of active ingredients, the functional analysis of combinatoric-chemical or combinatoric-biological synthesis-products, the functional genom-analyses, the evolutive biotechnology, the diagnostics, the proteom-analyses or in the investigation of material.